Toxicology

23. Additional information regarding your reproductive/teratological testing study provided in Amendment 4 is necessary. Please provide a systematic examination of a system, such as the skeletal system, to quantify the deformities in the test and control animals. If you are unable to provide this information, results of a new teratogenicity study of your finished, sterilized device may be necessary.

23 Response:

Mentor conducted an extended, one-generation reproductive and developmental study in CD rats, and did not detect any treatment or dose-related effects. We evaluated F0 females prior to mating and F1 animals through adulthood. F1 animals were evaluated for any potential systemic, developmental, neurobehavioral, immunologic, and/or reproductive effects from possible in utero and/or lactational exposure, or from any potential indirect effects of the gel on the F0 dams.

The F1 results of the study yielded the following conclusion: The F1 offspring from test gel-implanted dams showed no effect of treatment or dose on survival, growth, acquisition of developmental landmarks, learning and memory, FOB, hormone-mediated endpoints (e.g., anogenital distance, retained nipples and/or areolae in preweanling males, acquisition of puberty in retained postwean F1 males or females), systemic or reproductive organ weights, and gross or histopathologic lesions of organs in either F1 males or females.

We examined the F0 females every day until their scheduled necropsy at weaning of their F1 offspring. We examined the F1 offspring every day from birth to wean on pnd 21 and the retained postwean F1 offspring from weaning to scheduled adult necropsy. There were no noted external malformations or variations on any F0 female or F1 male and female. Please note that this was not a developmental toxicity/teratology study, so we did not examine the fetuses prior to expected parturition. We examined the newborn pups as soon after delivery as possible. Since there were no significant differences among groups for stillbirth/live birth indices, there is no evidence of pup external malformations/variations resulting in maternal infanticide in any group.

We necropsied the F0 females (25/group) at weaning of their litters (total 100 F0 females). We necropsied the culled F1 pups on pnd 4 (approx. 500 pups, 250/sex) when we standardized F1 litters. We necropsied nonselected F1 pups at weaning (a total of 178 F1 males, 37-50/group, and 179 F1 females, 42-48/group), and we necropsied all retained postwean F1 males and females at the adult necropsy (299 F1 males and 300 F1 females, 75/sex/group except for 74 F1 males at 10 ml/kg due to the number weaned). These necropsies correspond to detailed visceral examinations on approx. 1450 F1 offspring. We did not perform clearing, staining, or evaluation of the skeletal system of any F0 or F1 animal (as is done in a teratology study), but the

F1 animals survived and thrived, with no treatment- or dose-related effects on any behavioral tests, including those that required physical and mental competence.

Mentor believes that the study fully addresses the potential concern of reproductive and developmental, including teratogenesis, toxicity for this device. Furthermore, after FDA requested further testing from Mentor (letter dated March 11, 2003), Mentor submitted a copy of the proposed protocol for FDA's review and approval. Based on FDA's approval of this protocol, Mentor then conducted the study and subsequently submitted the results in February 2004.

Based on the aforementioned information, and the lack of any deformities identified or reported in the test report, Mentor believes that existing data are adequate.

In addition, please address the following concerns with this study:

a. Please explain the high amounts of erratic cycling seen in the treatment group

23a Response:

The results of examination for estrous cycling were not unexpected, based on the performing laboratory's experience, and are consistent with other multigenerational studies. For the F0 females, estrous cyclicity was evaluated during the last two weeks of the four-week prebreed period. There were no differences among groups for the number/percent of females cycling, number/percent of females with an abnormal cycle, or for the cycle length in days (text, p. 41; summary table 6, p. 96). For the F1 females, estrous cyclicity was evaluated for the last three weeks of the postweaning holding period. There were no differences among groups for the number/percent of females cycling or for cycle length in days. There were significant increases in the number/percent of females with an abnormal cycle at 3 (42.7%), 10 (38.7%), and 30 (48.6%) ml/kg versus the control value (24.3%) with no dose-response pattern (text, p. 49; summary table 30, p. 158). See Table 1 comparing the estrous cycle parameters for the F0 and F1 females. The data for the individual females evaluated are in Table A-6 for F0 females (Appendix VI, pp. 27-30) and Table A-45 for F1 females (Appendix VI, pp. 370-377).

Table 1. Comparison of Estrous Cyclicity Parameters in F0 and F1 Females

	F0 (ml/kg)				F1 (ml/kg)			
Parameters	0	3	10	30	0	3	10	30
No. females evaluated	25	25	25	25	75	75	75	75
No. (%) cycling	23 (92.0)	24 (96.0)	24 (96.0)	25 (100.0)	74 (98.7)	75 (100.0)	75 (100.0)	72 (96.0)
No. (%) with abnormal cycle	10 (43.5)	4 (16.7)	3 (12.5)	5 (20.0)	18 (24.3)	32 (42.7)*	29 (38.7)*	35 (48.6)**

	F0 (ml/kg)				F1 (ml/kg)			
Parameters	0	3	10	30	0	3	10	30
Cycle length in days (mean ± SEM)	4.1 (±0.2)	4.0 (±0.1)	3.8 (±0.1)	4.1(±0.1)	4.8 (±0.2)	5.4 (±0.3)	5.1 (±0.2)	5.0 (±0.3)

*, ** = $p<0.05$, <0.01; statistically significantly different from the concurrent control group value

For the F0 females at 25/group, evaluated for 14 days by a limited number of technical staff (25/group x 4 groups x 14 days/female = 1400 daily smears), the control mean percentage of females with an abnormal cycle was 43.5%, while the test gel implanted groups averaged 12.5-20% (Table 1). For the F1 females with 75/group and evaluated for 21 days, the control mean percentage of females with an abnormal cycle was 24.3% while the test gel implanted groups averaged 38.7-48.6% (Table 1). The abnormal cycles in the F0 females were predominantly prolonged estrus and proestrus. The abnormal cycles in the F1 females were predominantly prolonged diestrus. It is not uncommon that females evaluated for estrous cyclicity daily for 21 days, especially by a relatively large number of technical staff, necessitated by the large number of F1 females and the long duration (75/group x 4 groups = 300 females x 21 days/female = 6300 daily vaginal smears), go into pseudopregnancy from daily cervical stimulation for up to 10-12 days, evidenced by prolonged diestrus, with normal cycles before and after this period. This is what was observed in the F1 females. Based on the performing laboratory's experience, this situation was not unexpected and has been seen in a number of multigeneration studies that also require evaluation for estrous cyclicity for the last three weeks of the F0 and F1 prebreed periods. Examination of the individual animal data for F0 and F1 female cycle stages confirms this explanation. Technical staff performed the vaginal smears and identified the stage of each smear for each female and the Study Director was responsible for determining whether or not the females cycled, the presence or absence of abnormal cycles, and the mean duration of the estrous cycle for each female evaluated.

b. The trend in the F1 uterine weights suggests a possible estrogen effect with increasing dose. Please test this with a trend analysis to determine significance.

23b Response:

Statistical tests for trend include the Linear Trend Test by GLM procedures for parametric/homogenous continuous data (SAS Institute, Inc., 1999a,b,c,d,e; 2000; 2001) and the Cochran-Armitage Test for Linear Trend on Proportions and Frequencies (Cochran, 1954; Armitage, 1955; Agresti, 1990) for nominal scale measures. Also used are Jonckheere's test for trend (Jonckheere, 1954) for nonhomogenous/nonparametric data and robust regression methods for nonhomogenous data in the REGRESS procedure of SUDAAN®, Release 8 (RTI,

2001). All of these procedures for trend analysis heavily weight the control and high dose values to determine the presence or absence of trend. Since in this study the weights of the uterus with cervix and vagina (both absolute and relative to terminal body weight) of F1 adult females were statistically significantly increased at the top dose (30 ml/kg), the trend test would be significant by definition. Note that there were no biologically relevant or statistically significant effects on these parameters at 3 or 10 ml/kg. On pnd 21, there were no differences among groups for either absolute or relative weights of the uterus with cervix and vagina. Therefore, the *post hoc* analysis of the data for trend would not be informative. If there was a true "estrogenic effect," one would expect histopathologic confirmation of changes in the uterus with cervix and vagina, and there were no histopathologic findings in these or any other tissues, and changes in estrous cyclicity, and there were no effects on cycle length or percentage of females cycling. One would also expect an acceleration in acquisition of vaginal patency (puberty) but, in fact, absolute age at acquisition, was slightly but significantly delayed at 3 and 10 ml/kg and unaffected at 30 ml/kg. Age adjusted for body weight at acquisition (by analysis of covariance with body weight as the covariate) was slightly but significantly delayed only at 10 ml/kg and unaffected at 3 and 30 ml/kg. A delay in acquisition of preputial separation in males (puberty) is also observed in postwean offspring exposed to an estrogenic compound but, in fact, there were no effects on absolute or adjusted age of F1 males at preputial separation in this study. There were also no effects on anogenital distance at birth or on pnd 21 (weaning) in either sex in any group (an endpoint under endocrine control), no males with retained nipples on pnd 13 or 21, and no areolae in any group on pnd 21 (with areolae present on pnd 13, but no differences among groups; also under endocrine control), and no differences among groups in precoital interval or in gestational length (also influenced by endocrines). See Table 2 for a summary of endocrine-influenced parameters in males and females. There is, therefore, no evidence for "a possible estrogen effect with increasing dose" in this study.

Table 2. Female and Male Endpoints Under Endocrine Influence

Parameter		0 (ml/kg)	3 (ml/kg)	10 (ml/kg)	30 (ml/kg)
F0 female precoital interval (days)		3.8 ± 0.7	2.8 ± 0.3	2.4 ± 0.3	2.1 ± 0.2
F0 female gestational length (days)		22.0 ± 0.0	22.1 ± 0.1	22.1 ± 0.1	22.0 ± 0.0
Anogenital distance on pnd 0 (mm):					
F1 females:	Absolute	1.06 ± 0.02	1.05 ± 0.02	1.04 ± 0.01	1.02 ± 0.02
	Adjusted	1.06 ± 0.02	1.04 ± 0.02	1.03 ± 0.02	1.04 ± 0.02
F1 males:	Absolute	2.18 ± 0.02	2.22 ± 0.04	2.17 ± 0.03	2.13 ± 0.03
	Adjusted	2.17 ± 0.03	2.21 ± 0.03	2.15 ± 0.03	2.17 ± 0.03
No. nipples/male:					
F1 males:	pnd 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	pnd 21	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
No. areolae/male:					
F1 males:	pnd 13	0.28 ± 0.15	0.20 ± 0.08	0.43 ± 0.23	0.09 ± 0.04
	pnd 21	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Anogenital distance on pnd 21 (mm):					
F1 females:	Absolute	8.04 ± 0.19	8.80 ± 0.19*	8.78 ± 0.15*	8.43 ± 0.20
	Adjusted	8.05 ± 0.18	8.80 ± 0.19**	8.76 ± 0.18**	8.44 ± 0.21
F1 males:	Absolute	14.04 ± 0.28	14.10 ± 0.15	14.21 ± 0.18	14.32 ± 0.13
	Adjusted	13.98 ± 0.26	14.14 ± 0.14	14.13 ± 0.17	14.39 ± 0.14
Weight of uterus + cervix + vagina for F1 females					
Pnd 21:	Absolute	0.0868±0.0034	0.0899±0.0049	0.0847±0.0034	0.0794±0.0029
	Relative	0.1727±0.0063	0.1758±0.0070	0.1643±0.0056	0.1597±0.0060
Adult:	Absolute	0.6163±0.0194	0.6178±0.0225	0.6334±0.0230	0.7092±0.0284*
	Relative	0.2541±0.0082	0.2497±0.0103	0.2627±0.0092	0.2901±0.0119*
Age at puberty (days):					
F1 females:	Absolute	30.3 ± 0.2	31.6 ± 0.2***	31.7 ± 0.3***	30.6 ± 0.2
	Adjusted	30.6 ± 0.2	31.1 ± 0.2	31.5 ± 0.2***	31.0 ± 0.2
F1 males:	Absolute	42.4 ± 0.2	42.8 ± 0.3	43.0 ± 0.3	42.3 ± 0.3
	Adjusted	42.5 ± 0.2	42.7 ± 0.2	42.7 ± 0.2	42.6 ± 0.2

*, **, *** = $p<0.05$, <0.01, <0.001; statistically significantly different from the concurrent control group value

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c. Please provide the D4 and D5 levels of the lots used in the reproductive test.

23c Response:

The lot number of the devices used in the reproductive test was Lot #257949 (Catalog #354-3007, 300cc). Mentor has not measured - ---------- and D5 levels from this particular device lot; however, our ---- ---------- has measured the D4 and D5 levels in the raw material gel ---- ----------as 5 ppm and the D5 level was 17 ppm. Finished device gel D4 and D5 levels would be much less than these due to the heat history during manufacturing of the devices.

Updated Review and Assessment of the Low Molecular Cyclics D_4 and D_5

Low molecular weight cyclics from silicone gel-filled breast implants have been the focus of considerable attention in recent years. To fully address this issue, Mentor Corporation requested an independent review of pertinent information that has become available subsequent to the Institute of Medicine expert panel review completed in 1999. This independent review was conducted by Joseph V. Rodricks, Ph.D., DABT, an internationally-recognized, board-certified toxicologist specializing in risk assessment. His expert report is provided as Attachment 26 to this submission. Dr. Rodricks cites the findings of the IOM panel that "In general, there do not appear to be long-term systemic toxic effects from silicone gel implants or from unsuspected compounds in these gels or

elastomers..." Based on his review of the results of recently completed studies on silicone materials, Dr. Rodricks concludes that "these studies add to the body of evidence confirming the safety of silicone materials as used in silicone gel-filled breast implants and lend further support to the conclusions drawn by the panel in 1999." He also noted that "while substantial new data has become available since the completion of the IOM expert panel review, the findings do not affect the original conclusions made by the panel."

Along with earlier Dow Corning toxicology studies, the Rodricks expert report addresses for the first time some newly available results from Dow Corning 2-year chronic toxicity/carcinogenicity studies of D_4 and D_5 in rats, in addition to results from a two-generation reproductive toxicity study of D_4.

Based on the findings of Dr. Rodricks' review, updated risk assessments are provided below for both D_4 and D_5. The updated D4 risk assessment is based upon the same study (from Klykken and colleagues[1/]) and toxicity endpoint (reversible liver weight increases) as before, however, a correction factor for the 5 days per week (vs. 7 days per week) inhalation exposure over the course of the study has been incorporated, resulting in a NOAEL equivalent to a dose of 0.75 mg D_4/kg/day (as compared with the previous NOAEL of 1.05 mg D_4/kg/d). The updated D_5 risk assessment is based on a recently completed Dow Corning 2-year chronic toxicity/carcinogenicity study in rats. The most sensitive toxicity endpoint in this study was uterine endometrial tumors observed in rats exposed for 12 to 24 months to D_5 via inhalation, with no other adverse effects reported in this study. The resulting NOAEL for D_5 corresponds to an estimated daily dose of 5.4 mg D_5/kg/day (as compared with the previous NOAEL of 14 mg D_5/kg/day).

Updated D_4 Risk Assessment

The potential amount of D_4 present in a whole device was 0.47 µg/g, or 785 µg of D_4 per two devices, for an estimated reasonable worst-case daily exposure of 26 µg D_4. For D_4, the most sensitive toxicity endpoint observed in rodent bioassays has been a dose-related increase in liver weights (reversible following removal of exposure). In the inhalation toxicity study of D_4 reported by Klykken and colleagues, rats were exposed to vapor concentrations of 0, 7, 20, 60, 180 or 540 ppm and the lowest-observable-adverse-effect level (LOAEL) was found to be 0.24 mg/L (20 ppm) and the no-observable-adverse-effect level (NOAEL) to be 0.085 mg/L (7 ppm) for an exposure period of 6h/day, 5d/wk for 28 days in Fischer 344 rats. In a study evaluating the retention, distribution, metabolism and

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1/ Klykken, P.C., T.W. Galbraith, G.B. Kolesar, P.A. Jean, M.R. Woolhiser, M.R. Elwell, L.A. Burns-Naas, R.W. Mast, J.A. McCay, K.L. White, Jr., and A.E. Munson. 1999. Toxicology and humoral immunity assessment of octamethylcyclotetrasiloxane (D_4) following a 28-day whole body vapor inhalation exposure in Fischer 344 rats. *Drug Chem. Toxicol.* 22(4):655-677.

excretion of D_4 in Fischer 344 rats, Plotzke et al.[2/] reported that 5 to 6 percent of an inhaled dose is retained. Assuming a body weight of 350 g, a minute ventilation rate for rats of 240 mL,[3/] and 5 percent retention,[4/] and factoring in the 5d/wk vs. 7d/wk exposure regiment the NOAEL is equivalent to approximately 0.75 mg D_4/kg body weight/day, equivalent to 45,000 µg/day for a 60 kg adult. This NOAEL is 1,700-fold higher than the estimated reasonable worst-case daily exposure from Mentor silicone gel-filled implants.

Updated D_5 Risk Assessment

The potential amount of D_5 present in a whole device was 2.47 µg/g, or 4,125 µg of D_5 per two devices, for an estimated reasonable worst-case daily exposure of 138 µg D_5. For D_5, the most sensitive toxicity endpoint observed in rodent bioassays has been an increase in uterine endometrial tumors in rats exposed to D_5 for 12 to 24 months.[5/] In this study, rats were exposed to vapor concentrations of 0, 10, 40 or 160 ppm for 6h/d, 5d/wk for up to 24 months, and no adverse effects other than the uterine endometrial tumors in the high dose group were observed. Assuming a body weight of 350 g, a minute ventilation rate for rats of 240 mL,[6/] and 5 percent retention (based on the D_4 data of Plotzke and colleagues), the NOAEL of 40 ppm is equivalent to approximately 5.4 mg D_5/kg body weight/day, equivalent to 324,000 µg/day for a 60 kg adult. This NOAEL is 2,300-fold higher than the estimated reasonable worst-case daily exposure from Mentor silicone gel-filled implants.

2/ Plotzke, K.P., S.D. Crofoot, E.S. Ferdinandi, J.G. Beattie, R.H. Reitz, D.A. McNett and R.G. Meeks. 2000. Disposition of radioactivity in Fischer 344 rats after single and multiple inhalation exposure to [^{14}C]Octamethylcyclotetrasiloxane ([^{14}C]D_4). *Drug Metab. Dispos.* 28(2):192-204.

3/ Hayes, A.W., Ed. 2001. *Principles and Methods of Toxicology, 4th Edition*, Philadelphia: Taylor & Francis; p. 206.

4/ Plotzke, K.P., S.D. Crofoot, E.S. Ferdinandi, J.G. Beattie, R.H. Reitz, D.A. McNett and R.G. Meeks. 2000. Disposition of radioactivity in Fischer 344 rats after single and multiple inhalation exposure to [14C]Octamethylcyclotetrasiloxane ([14C]D4). *Drug Metab. Dispos.* 28(2):192-204.

5/ Dow Corning. 2003. Decamethylcyclopentasiloxane (D5): A 24-month combined chronic toxicity and oncogenicity whole body vapor inhalation study of Decamethylcyclopentasiloxane (D5) in Fischer-344 rats. CD Study No. 9346. (Unpublished). February 3, 2003. TSCA Section 8(e) Notification of Substantial Risk: Decamethylcyclopentasiloxane. 6 pp. (Summary).

6/ Hayes, A.W., Ed. 2001. *Principles and Methods of Toxicology, 4th Edition*, Philadelphia: Taylor & Francis; p. 206.

24. Your response to item 3 of our March 11, 2003 letter for M020018/M1 raised additional questions. Please respond to the issues below regarding your 1998 carcinogenicity study.

a. In Table 9 (p.91), summarizing the non-neoplastic histology, implant material was found in the capsule in only half the animals at the highest dose and in only 12% of the animals at the low dose. At the control site, implant material was found in the capsule in only 6 of 238 sites examined. Please explain why implant materials were so difficult to find in the capsule for both the silicone gel and the control material.

Response to 24.a:

The difficulty in finding implant materials in the capsule for both the silicone gel and the control material is attributable to more than one factor based on information presented in the pathology report for this study and from previously conducted studies. A factor common to the expected solid-state tumorigenesis response for both control and implant materials was the reported observation (Study No. 7088, Appendix B, page 9) that "[i]n a number of animals, particularly in the groups with the largest implants for both the gel and polyethylene implants, sarcomas completely surrounded the implants and fibrotic capsules could not be identified."

The pathologist who supervised the necropsies for this study was Dawn G. Goodman, VMD, (formerly with the National Cancer Institute and Pathco, currently with the Department of Pathology at Covance), who is an experienced, well-known and respected board-certified veterinary pathologist and has served on and/or chaired many NTP Pathology Working Groups and EPA Cancer Risk Assessment Peer Review Panels for a number of potentially carcinogenic materials. Dr. Goodman recalls the great difficulty in locating the implant material, particularly for controls, in these animals (personal communication with Roger Wixtrom, Ph.D., DABT, March 2004). In the pathology report (Appendix B, page 9), Dr. Goodman provided the primary explanation for why the control material was so difficult to identify: "the implant consisted of a thin, small translucent disk which was difficult to identify at necropsy."

Another basis for the difficulty at necropsy of visualizing the implanted silicone gel material is well understood, with similar experience having been reported in other animal species similarly implanted with silicone gel. Following implantation of free gel (not surrounded by an elastomer shell), the gel is encapsulated by a fibrous capsule with subsequent ingrowth of connective tissue into the gel mass resulting in progressive subdividing and compartmentalization of the gel[7/] that can make visualization of the originally implanted gel mass

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7/ "At 30 days post-implantation there was evidence of subdivision of the large mass of gel by trabeculae of connective tissue, resulting in the isolation of small globules of gel in the surrounding tissue. This process was much more advanced by 90 days after implantation with the

difficult upon necropsy. Based upon results of such animal experiments conducted in the 1970s and 1980s by Dow Corning, a decision was made early on not to pursue further the idea put forth by some plastic surgeons of implanting gel without a shell for breast augmentation and reconstruction (the progressive subdividing and compartmentalization of the gel led to a very firm implant without the desired soft feel typical of implants composed of gel surrounded by an elastomer shell).

b. Mineralization was seen in only 1 of 238 control implantation site capsules, but in 62 of 291 test implantation site capsules at site 1 and in 43 of 298 capsules at implant site 2. The local effects of implants are important effects. Please propose some potential causes of the mineralization by the implants, provide evidence to support the cause, and relate this to the local adverse events such as contracture.

24b Response:

These incidence rates for remineralization correspond to 0.4% (polyethylene controls), 21% (gel implant site #1) and 14% (gel implant site #2). The relative absence of calcification (the most frequent from of mineralization observed *in vivo*) for the polyethylene control implants is likely based on the reduced propensity of calcium salts to bind to this material.

Shumakov et al.,[8/] in a study of biomaterial calcification, found that "the degree of calcification for silicone rubber was greater than that for polyurethane and polyethylene." (Such observations are also consistent with typical marketing claims of "no calcification" commonly made for polyethylene pipes in various (non-medical) applications.) The etiology of calcification of biomaterials and breast implants is not well understood.[9/] Regardless of the site (tissue), the common pathway is the formation of crystalline calcium phosphate mineral. The process has two major stages: initiation in membrane-bound vesicles derived from degenerating or aging cells, and propagation of crystal formation. Collagen

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bulk of the implant area occupied by connective tissue containing divided portions of the gel" (Dow Corning Corporation. 1983. Ninety-Day Implant Study of Dow Corning Q7-2167/68-Gel (Q7-2159 A) in New Zealand White Rabbits. Dow Corning Tox. File No. 2476-8.0 "By 42 days after injection of the gel, ridges of fibrous tissue had begun to develop from the inner aspect of the capsule and project into the gel mass. Behind each of these ridges there were increased numbers of inflammatory cells. This process of tissue ingrowth continued through the 84 and 168 day observation periods. By 168 days the gel mass was fragmented into smaller masses by a network of tissue ingrowth" (Dow Corning Corporation. 1986. Dow Corning Q7-2159A Gel Injected Subcutaneously into Rats: Microscopic Observations. Dow Corning Report #1986-I0740-179).

8/ Shumakov, V.I., I.B. Rosanova, S.L. Vasin, L.A. Salomatina, and V.I. Sevastianov. 1990. Biomaterial calcification without direct material-cell interaction. *ASAIO Trans.* 36(3):M181-M184.

9/ Peters, W. and D. Smith. 1995. Calcification of breast implant capsules: Incidence, diagnosis, and contributing factors. *Ann. Plast. Surg.* 34(1):8-11.

enhances the rate of crystal growth.[10/] Shumakov and colleagues also proposed, based on their findings, that biomaterial calcification may develop without direct contact of biomaterials with cells.

Mineralization (likely primarily calcification) has been observed in other long-term animal studies of implanted silicone polymer. For example, in Mentor's Low Bleed pulverized shell chronic toxicity/carcinogenicity study, mineralization at the implant site was observed microscopically. Nine (9) rat implant sites (about 15%) showed focal or multifocal mineralization in both the smooth and textured gel implant shell groups (Saline PMA Biological Section, Vol. V.C.3, no. 17 or 31, pgs. 5327 and 5340). For comparison, in Mentor's Saline shell groups, 50% - 60% of the implant sites showed evidence of mineralization.

As discussed in the review summary of calcification below, the incidence of calcification in women with breast implants is influenced by the site of implantation (*e.g.,* submuscular vs. subglandular) suggesting the possible involvement of physical factors. Such factors offer a potential explanation for the relative absence of calcification in the high-dose group which was implanted with 4.4- to 20-fold higher volumes of gel than the mid and low-dose groups, respectively.

With respect to the potential clinical significance of these animal study findings, calcification of breast implant capsules, as discussed below, is generally not of clinical significance, although it may exacerbate symptoms of capsular contracture.

Calcification and Silicone Breast Implants: A Review Summary[11/]

Calcification of a biomaterial occurs when calcium salts are deposited in the tissue capsule surrounding the implanted device. Calcification occurs in association with a wide variety of implanted prostheses, including breast implants, heart valves, vascular grafts, and soft contact lenses, as well as in the mature breast tissue of women that have not undergone breast surgery.

Calcification of the fibrous capsule that may occur following implantation of breast prostheses is generally not of clinical significance,[12/] although it may

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10/ Cotran, R.S., et al. 1994. *Robbin Pathologic Basis of Disease.* 5th ed. Philadelphia: W.B. Saunders Company.

11/ Updated version of calcification information previously provided to FDA for silicone gel-filled breast implants.

12/ Ganott, M.A., K.M. Harris, Z.S. Ilkhanipour, and M.A. Costa-Greco. 1992. Augmentation mammoplasty: Normal and abnormal findings with mammography and US. *Radiographics* 12:281-295; O'Boyle, M.K., R.J. Wechsler, E.F. Conant, A.S. Lev-Toaff, and J. Sagerman. 1994. Breast implants: incidental findings on CT. *Am. J. Roentgenol.* 162:311-313.

exacerbate symptoms of capsular contracture.[13/] Concern has been raised, however, that calcification may interfere with tumor detection, because microcalcifications are considered a hallmark of malignant breast disease. Although clinicians have recommended pre- and post-surgical mammograms for augmentation patients to assist in distinguishing post-operative findings from calcification associated with malignancy, benign calcification resulting from surgery is generally considered distinguishable from malignant-type calcification.[14/] Further, no published reports were identified that document any actual occurrences of missed or delayed diagnoses attributable to capsular calcification.

The etiology of calcification in breast implants is not well understood. Among the factors that have been associated with a greater occurrence of calcification in breasts following augmentation or reconstructive surgery are postoperative infection and inflammation or calcium supplementation, implants with Dacron patches, length of time since implantation, and subglandular (versus submuscular) placement of implants. Implant generation is also strongly associated with the incidence of capsular contracture, affecting 100% (28/28; mean duration 17.6 yr) of first generation implants (which had Dacron patches), 9.8% (34/348; mean duration 16.0 yr) of second generation implants, and 0% (0/28; mean duration 4.2 yr) of third generation implants in one study of explanted devices.[15/] Tissue silicon levels do not correlate with the presence or absence of calcification.[16/]

Examination of the occurrence of calcification in women with augmentation or reconstructive mammoplasty through retrospective reviews of screening mammograms suggests incidence rates ranging from 5 to 26 percent.[17/] In studies of patients whose implants had been removed (explanted prostheses), the incidence of calcification was reported to range from 9 to 16 percent.

13/ Young, V.L., T. Bartell, J.M. Destouet, B. Monsees, and S.E. Logan. 1989. Calcification of breast implant capsule. *South. Med. J.* 82:1171-1173.

14/ Eklund, G.W. 1991. Diagnostic breast imaging in plastic surgery of the breast. In: Plastic and Reconstructive Surgery of the Breast. R. Barrett Noone, editor. B.C. Decker, 48-69.

15/ Peters, W., K. Pritzker, D. Smith, V. Fornasier, D. Holmyard, S. Lugowski, M. Kamel and F. Visram. 1998. Capsular

16/ McConnell, J.P., T.P. Moyer, D.E. Nixon, P.L. Schnur, D.R. Salomao, T.B. Crotty, J. Weinzweig, J.B. Harris and P.M. Petty. 1997. Determination of silicon in breast and capsular tissue from patients with breast implants performed by inductively coupled plasma emission spectroscopy. Comparison with tissue histopathology. *Am. J. Clin. Pathol.* 107(2):236-246.

17/ Jensen, S.R. and J.K. Mackey. 1985. Xeromammography after augmentation mammoplasty. *Am. J. Roentgenol.* 144:629-633; Dershaw, D.D. and T.A. Chaglassian. 1989. Mammography after prosthesis placement for augmentation or reconstructive mammoplasty. *Radiology* 170:69-74.; Destouet, J.M., B.S. Monsees, R.F. Oser, J.R. Nemecek, V.L. Yound, and T.K. Pilgram. 1992. Screening mammography in 350 women with breast implants: Prevalence and findings of implant complications. *Amer. J. Roent.* 159:973-978; Ganott, M.A., K.M. Harris, Z.S. Ilkhanipour, and M.A. Costa-Greco. 1992. Augmentation mammoplasty: Normal and abnormal findings with mammography and US. *Radiographics* 12:281-295.

Calcification is not a phenomenon unique to breast implants. It occurs in 11 to 53 percent of women who underwent breast reduction procedures.[18/] In the breasts of women who have not undergone any breast surgery, the prevalence of benign calcifications increases progressively with age from 8 percent in women 25 to 29 years old up to 86 percent in women 75 to 79 years old.[19]

25. In response to item 10 of our March 11, 2003 letter for M020018/M1, regarding the dose of devices tested expressed in square centimeters, you stated that extracts of the devices were used. Please provide complete descriptions of the sample preparations so the exposures can be quantitatively assessed for the cytotoxicity, cutaneous reactivity, hemocompatibility, and acute systemic toxicity testing.

25 Response:

We received a letter from NAMSA explaining that for the test sample preparations the entire device was extracted. The letter also contains details on exactly how the sample preparations were performed. A copy of this letter in included in Attachment 27.

Silicone Gel Bleed Testing

26. Silicone gel bleed, which is the diffusion of gel constituents (e.g., low molecular weight silicones) through an intact shell, appears to occur continuously for silicone gel-filled breast implants. To address silicone gel bleed, you provided extended ASTM F703 testing and a gel loss analysis.

The ASTM F703 test methodology quantifies the extent of gel bleed. However, as you stated, the results from this testing has limited clinical correlation because the ASTM F703 test method was established for the purpose of allowing comparison between device models rather than quantifying in-vivo gel bleed. In addition, the ASTM F703 test method was not established to identify and quantify the gel bleed constituents. Thus, FDA does not believe that this test methodology provides adequate data to address gel bleed for the purposes of a PMA.

The gel loss analysis in Section 8.5 of the PMA was intended to determine the rate of gel loss over time in-vivo from intact explanted devices. The gel loss was

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18/ Abboud, M., J. Vadoud-Seyedi, A. De Mey, M. Cukierfajn, and M. Lejour. 1995. Incidence of calcification in the breast after surgical reduction and liposuction. *Plast. Reconstr. Surg.* 96:620-626; Mitnick, J.S., D.F. Roses, M.N. Harris, and S.R. Colen. 1990. Calcifications of the breast after reduction mammoplasty. *Surg. Gynecol. Obstet.* 171:409-412; Brown, F.E., S.K. Sargent, S.R. Cohen, and W.D. Morain. 1987. Mammographic changes following reduction mammaplasty. *Plast. Reconstr. Surg.* 80:691-698.

19/ Stomper, P.C., D.J. D'Souza, P.A. DiNitto, and M.A. Arredondo. 1996. Analysis of parenchymal density on mammog

determined based on a comparison of the explant weight to the design weight specifications. One major weakness of your gel loss analysis is that you based your rationale of why the minimal weight change was not due to diffusion of materials entering the device and mixing with the gel filler on the visual appearance of the gel. Another major weakness is that you had no unimplanted control devices for comparison purposes. FDA does not believe that an accurate assessment of overall gel bleed over time can be made on these data as a result of these study weaknesses. More importantly, FDA believes that the use of explanted devices to assess gel bleed is problematic because the in-vivo and in-vitro environmental conditions for explanted devices are variable and unknown.

As stated in our January 2004 breast implant guidance document, we believe that information regarding the amount and identity of gel bleed constituents should be provided. Neither your ASTM F703 testing or gel loss analysis provide this information. Therefore, please provide the identity of the gel bleed constituents (including the platinum species or other catalysts) and the rate that these gel constituents bleed out over time. To address this item, you should consider a new gel bleed bench test based on a protocol that mimics in-vivo conditions (e.g., incubate the breast implants in a lipid-rich medium prior to testing and conduct testing in a physiologic environment). This information is needed to provide adequate labeling for women who may be considering breast implants.

26 Response:

Gel Bleed Test Results (CP 246, CP 246 Addendum I in Attachment 28, CP 411, and CP 411 Addendum I in Attachment 29)

Mentor has performed new *in vitro* bleed experiments to determine the identity and diffusion rate of potential bleed materials (see Report CP 246 in Attachment 28). This testing utilized an intact device in physiological media (*i.e.*, porcine serum), which was selected to simulate the composition, including lipid content, of the extracellular fluid within the fibrous capsule that is in direct contact with the implant in the patient.[20/,21/] Data for low molecular weight dimethylcyclosiloxane, linear siloxane, and vinylterminated linear siloxane diffusion (by gas chromatography/mass spectroscopy), and platinum diffusion (by inductively coupled plasma/mass spectroscopy) from a device into serum were collected. Based upon the results, only D4, D5, D6, and platinum exhibited measurable diffusion into the serum over a 120-day period at 37°C; however, a time-dependent trend was evident for platinum, but not the siloxane compounds. All diffusion of these compounds into serum ceased by

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20/ Ostrowska, E., Gabler, N.K.. Sterling, S.J., Tatham, B.G., Jones, R.B., Eagling, D.R., and Dunshea, F.R. "Consumption of Brown Onions (allium Cepa Var. Cavalier and Var. Destiny) Modulates Blood Lipids, Haematological and Haemostatic Variables in Healthy Pigs," *Br. J. Nutr.*, 91(2), 211, 2004.

21/ Tietz, Textbook of Clinical Chemistry, Edited by Burtis, C.A. and Ashwood, E. R. 3rd Edition, p. 826-827 (1999).

120 days. The largest total amount of low molecular weight siloxane compound (D_5) diffusing into the serum from a 125cc Smooth Round Moderate Profile Gel-filled device was only 2.8 μg. Only a total of 4.1 μg of platinum was detected in the serum. These data suggest that the amount of silicone and platinum diffusing from intact gel-filled devices into physiological surroundings *in vivo* is very low, i.e., in the microgram range.

These *in vitro* bleed data strongly support Mentor's previous finding that intact explanted gel-filled devices have virtually no detectable weight loss due to gel bleed, even after as long as fifteen years of implantation. Bleed from these devices into physiological surroundings is very low compared to the weight of these devices.

Extensive testing, including the new gel bleed studies, demonstrated that there is little gel bleed from the device. Thus, the conclusions reached in the weight loss report included in our original PMA submission are supported by the following additional information provided in this PMA amendment: an amended copy of that explant weight loss report (Report M 054 in Attachment 30), new gel bleed studies, and a report providing data to demonstrate that gel-filled devices implanted in patients for as long as about nine years did not take up appreciable amounts of water, protein, or lipids (the most common biological materials surrounding the implant) into the device shell or gel filler (see attached report CP 411 in Attachment 29, Explant Testing: determination of Moisture, Protein, and Fat). Three explanted devices (implanted for 3.5, 6.4, and 8.9 years) were analyzed for water, protein, and lipid content.. At most, biological materials with a total weight of only approximately one-third of a gram for an 800cc device were taken up over about nine years. Therefore, one can only conclude that the lack of noticeable device weight change over the nine to fifteen years of implantation presented in the original device weight loss report was due to the relative lack of gel bleed from the device.

Originally, Mentor did not test unimplanted control devices as part of the device weight loss study because such controls would not have provided any useful information to help understand whether the explant bleed rate determination was meaningful or an artifact. Any weight loss from an unimplanted device packaged for at least nine years would have represented gel bleed onto or into the packaging material for that period of time. How these control bleed rates would be expected to compare with the *in vivo* bleed rates is not known, so that one cannot meaningfully interpret any differences observed between unimplanted control and *in vivo* bleed rates. (Recall that Mentor has already presented data in the Chemistry Module indicating that device bleed into one polymeric material, *i.e.*, a silicone disc, is much different from *in vivo* bleed rates.) If the packaged unimplanted control devices (stored for at least nine years on a shelf) had shown an appreciable increase in weight that increase could only have been due to water uptake from humidity. Since water content was measured in some of the explanted devices and was found to be almost nonexistent, unimplanted controls are not necessary to account for this possibility.

However, subsequent to the original device gel bleed report, as part of a project investigating semivolatiles compounds in gel, the weight of an unimplanted Mentor smooth and three unimplanted textured control d---------- ---- - ---------- r at least nine years was measured at ---------- --------- ---- - ---------- ---------- --- Based upon their measured weight---------- ----------ed device weight specification, these devices were 102 – 103% of their nominal specification weights. These weight percentages are within the range of those seen with the explanted devices in the original device weight loss report. Based upon these unimplanted control data and the knowledge that virtually no water, protein, or lipids have entered the device, the original device weight loss data strongly suggest that in the worst case only very small amounts of gel bleed may leave the device *in vivo* (*e.g.*, a fraction of a gram from an 800cc device) even over an implantation period as long as nine years.